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Electroanalytical determination of reduced-glutathione in biological samples

Ebru Kuyumcu Savan
Analytical Chemistry Division, Faculty of Pharmacy, İnönü University, Malatya, Turkey

Abstract

Aim: In this study, it was aimed to determine the reduced Glutathione (GSH) at the modified sensor with a simple and reliable electroanalytical method. Material and Method: Cyclic voltammetry and differential pulse voltammetry techniques were used for the determination of reduced-GSH. And the modified sensor was fabricated by dropping single-walled carbon nanotube (SWCNT) dispersion onto the surface of the glassy carbon electrode. Results: The determination of reduced-GSH was accomplished at SWCNT modified sensor. The relationship between the current responses of the sensor and the concentrations of the reduced-GSH (1.0×10^{-8} - 5.0×10^{-8} M) showed excellent linearity and the detection limit was calculated as 75 nM. Discussion: Determination of trace amounts of reduced-GSH in urine samples was successfully applied at the modified sensor. The results showed that modification of the glassy carbon electrode with SWCNT could provide a new strategy for determining the concentration of GSH in physiological solutions.

Keywords

GSH; Electrochemical Sensor; Voltammetry

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Corresponding Author: Ebru Kuyumcu Savan, Faculty of Pharmacy, Analytical Chemistry Division, İnönü University, Malatya, Turkey.
T.: +90 4223411216 F.: +90 4223411217 E-Mail: ebru.savan@inonu.edu.tr
ORCID ID: 0000-0002-8851-0907

Introduction

Glutathione (GSH) is the most abundant intracellular thiol with the concentration in the millimolar range (1–10 mM) [1-3]. GSH plays a very important role in regulating various physiological and pathological processes [1,4]. It has been reported that GSH serves many cellular functions, including xenobiotic metabolism, intracellular signal transduction, and gene regulation [3]. In addition, the intracellular redox activity of GSH continues with its antioxidant properties[5, 6]. Abnormal levels of GSH can lead to diabetes mellitus, Alzheimer's disease, atherosclerosis and other diseases [6-8]. More importantly, changes in the concentration of GSH in biological tissues are used as a marker for certain disorders [4]. In view of the important biological and clinical significance for GSH, it has thus spurred strong interest in developing useful chemical materials for quantitatively evaluating the level of intracellular GSH [4,8].

Glutathione is a tripeptide which can be synthesized in the liver using no genetic information [9-12]. It is a major antioxidant which protects cells from oxidative damage by reacting with free radicals and peroxides [7,9,10]. Glutathione exists in tissues in two different types which stay in balance in tissues: reduced GSH and oxidized glutathione (GSSG) [13-15]. In the physiological process of GSH, the conversion to GSSG occurs by the glutathione peroxidase enzyme with selenium. Oxidized glutathione reduces the oxidized form of GSH by an NADPH-dependent flavoenzyme GSH reductase [15]. It is important to detect GSH using a reliable method with good sensitivity and selectivity. Due to its electrochemically oxidizable property [16, 17], it has become important to develop an effective electrochemical sensor for the determination of GSH. For this reason, a simple and reliable electroanalytical method and a sensor modified with single-walled carbon nanotube (SWCNT) were developed to determine the reduced-GSH. To the best knowledge of this work, this is the first study of the determination of GSH at SWCNT modified sensor in the literature.

Material and Method

Reagents and Apparatus

Reduced GSH was purchased from Merck, and N, N-dimethylformamide (DMF) was from Sigma. Single-walled Carbon Nanotube (SWCNT) (diameter: *<2 nm*, EC: >100 S/cm, length: 5-30 μm, purity >95%, surface area: 380 m^2g^{-1}) was supplied from © Grafen Inc. Stock GSH solutions were freshly prepared in ultra-pure water for daily. Na_2HPO_4, KH_2PO_4 (Carlo Erba), KCl (Merck) and NaCl (Merck) were dissolved for 0.10 M phosphate buffer solution (PBS). Milli Q (Millipore, 18.2 MΩ.cm) ultra-pure water was used during the preparation of solutions.

All the electrochemical operations (Cyclic voltammetry (CV) and Differential pulse voltammetry (DPV) were carried out by a BAS (Bioanalytical Systems, Inc.) 100 W electrochemical analyzer. The three electrode system was used consisting of a glassy carbon working electrode (GCE) (geometric area: 6.85 mm^2, CHI), an Ag/AgCl reference electrode (CHI112) and a Pt disc auxiliary electrode (CHI). The pH was measured with a Jenway 3010 pH meter.

Measurement of GSH in urine samples

Urine samples were diluted 10 times with 0.1 M PBS (pH 7.4) without any additional pretreatment. After that, 100, 200 and 300 μM of reduced GSH added to 10 mL of this solution in an electrochemical cell for electroanalytical measurements. Three multi-run DPV measurements were performed in these samples prepared with three replicates at the modified sensor. The standard addition method was applied to the urine samples by spiking of reduced GSH.

Preparation of Modified Sensors

SWCNT dispersions were prepared with N,N-dimethylformamide (DMF) solution at the concentration of 1.0% (mg/μL). SWCNT/DMF dispersions were sonicated for 4 h before dripping onto the GCE surface. Modification of GCEs was carried out by dripping of 10 μL of SWCNT dispersions on the surface of bare GCE. And then SWCNT/GCE modified sensor was dried overnight.

Results

Electroanalytical determination of 10 μM reduced GSH was done in PBS (pH 7.4) at SWCNT/GCE modified sensor with DPV and CV techniques (Figure 1). In order to obtain a calibration graph, the solutions of reduced GSH were analyzed by the DPV technique at SWCNT/GCE modified sensor. The concentrations of reduced GSH were changed from 1 x 10^{-8} M to 5 x 10^{-8} M. The calibration curve was generated by plotting the current values against the concentrations (Figure 2). The results showed that the anodic peak currents of GSH were linear at the concentration range of 0.01 μM to 0.05 μM. The calibration equation was Ipa (μA) = 0.0956C (μM) + - 0.0166 with correlation coefficients (R^2) as 0.9909. Excellent correlation was found between the concentrations of GSH and peak current densities.

Validation parameters were shown in Table 1. The sensitivity and the detection limit (LOD) of GSH were found from the slope of the calibration curve. The limit of detection (s/*m* = 3), where s is the standard deviation of the peak currents (for five runs)

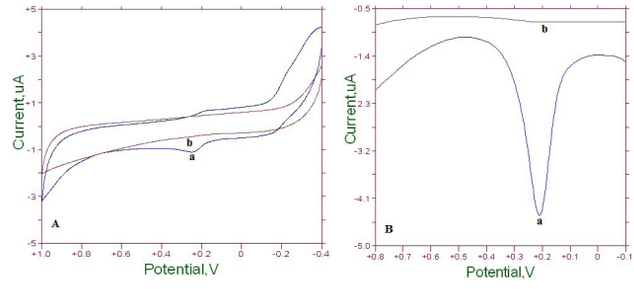

Figure 1. (A) CVs of GSH and (B) DPVs of GSH in PBS (pH 7.4) at (a) SWCNT/GCE modified sensor and (B) bare GCE vs. Ag/AgCl.

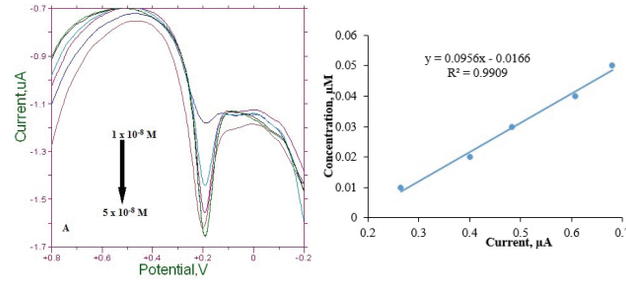

Figure 2. (A) DPVs of increasing concentrations of GSH (1 x 10^{-8} - 5 x 10^{-8} M) in PBS (pH 7.4) at SWCNT/GCE modified sensor (B) Calibration plot of GSH obtained from DPV results.

and *m* is the slope of the calibration curve, was 0.75 nM and the sensitivity was 0.0956 μA/μM. Three replicate solutions of urine samples containing reduced GSH were analyzed using the DPV technique at SWCNT/GCE modified sensor. GSH concentrations in real samples were calculated from the calibration curve equation by using the obtained current values. The recovery values obtained from the GSH analysis in real samples showed the accuracy and reproducibility of the sensor in Table 2. The proposed method was satisfactorily performed to real samples.

Table 1. Regression Data of the Calibration Line for Quantitative Determination of GSH in PBS at modified sensor by DPV Technique

Parameters	Results
Equation of calibration plot	I (μA) = 0.0956 C(μM) - 0.0166
Measured potential (mV)	210
Linear concentration range (M)	1.0×10^{-8} - 5.0×10^{-8}
Slope (μA. μM^{-1})	0.0956
Intercept (μA)	0.0166
Correlation coefficient	0.9909
Limit of Detection (μM)	0.75
Limit of Quantification (μM)	2.5

Table 2. Analytical results obtained by spiking the GSH to urine sample at modified sensor

Spiked, μM	Found, μM (mean±SD)	Recovery, %	RSD*, %
100	99.03 ± 0.73	99.03	0.737
200	198.72 ± 0.86	99.36	0.433
300	298.84 ± 0.95	99.61	0.318

* RSD: Relative standard deviation

Discussion

The effect of supporting electrolyte and pH, which are one of the parameters that can affect the voltammetric behavior of GSH, play an important role. Selecting a suitable support electrolyte creates a conductive medium for sensing the analyte in the solution medium to be analyzed and the sensitivity of the sensor increases. The choice of support electrolyte and pH depend on its resolution, dissociation degree, and nucleophilic character [18,19]. For this purpose, the DPV responses of GSH were investigated in PBS at pH 5.0 – 8.0. There was an increase in peak currents up to pH 7.4, but a decrease in peak currents after pH 7.4. It was found that the anodic peak potential of GSH shifted negatively with the increase of pH value, and the anodic peak current decreased due to the participation of two protons in the oxidation process [20, 21]. Consequently, subsequent electroanalytical studies were performed at PBS pH 7.4 as the supporting electrolyte medium. Furthermore, the maximum response at pH 7.4, which is physiological pH, is promising in terms of working with real samples.

The electrochemical determination of GSH was studied at GCE modified with SWCNT and bare GCE by CV and DPV method. The oxidation peak current of DPV response was selected as the analytical signal of GSH. The amperometric sensor displayed excellent electrochemical performance to the oxidation of reduced GSH. The GSH oxidized and reduced at SWCNT/GCE modified sensor by CV techniques at potentials of nearly 247 mV and 177 mV, respectively. GCE could not show any reduction/oxidation peak at bare GCE. GSH oxidized at nearly 212 mV (3.198 μA) by DPV method. The quantification of GSH was successfully achieved with these techniques.

The five successive tests at the same SWCNT/GCE modified sensor and different modified sensors imparted almost the same electroanalytical response with the recoveries of 99.12% and 98.65%, respectively. These results demonstrated that SWCNT/GCE modified sensor has excellent reproducibility and repeatability. Furthermore, ten cycles of the GSH oxidation currents at the SWCNT/GCE modified sensor was exhibited great stabilization by 0.024% RSD. Additionally, very low concentrations of GSH were perfectly determined at the modified sensor and a very low detection limit, such as 0.75 nM, was obtained. In order to determine the analytical applicability and accuracy of the modified sensor, urine samples were analyzed using SWCNT modified sensor. The satisfactory recovery values showed that the proposed sensor could be easily used to identify GSH in physiological fluids.

Several studies have shown the quantification of GSH at different modified sensors. However, the voltammetric response properties obtained in this work (including linear range and detection limit) are better than similar studies from the literature [1,3,5,8,22-32]. As a result, of a comparison between voltammetric response properties of the various modified electrode and proposed modified sensor, higher detection limit values are obtained at the corresponding sensors. This proved that the sensor used in this study could detect lower GSH quantities.

Conclusion

GSH does not present well-known redox response at the bare glassy carbon electrode. Surface modification of the electrode with SWCNT allows analyzing the alteration of the sensor electrochemical response. Voltammetric studies of GSH at the surface of the modified electrode have shown adsorption-like behavior in which, the GSH is linked to the composite. Determination of very low concentrations of GSH in physiological liquids was achieved by the electroanalytical method at the modified sensor with high recovery values. The results suggested that the SWCNT modification of electrodes may provide a new strategy for GSH concentration determination in physiological solutions. Therefore, the modified sensor can be promising to be used in routine analyses.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Yuan B, Zeng X, Xu C, Liua L, Maa Y, Zhanga D, et al. Electrochemical modification of graphene oxide bearing different types of oxygen functional species for the electro-catalytic oxidation of reduced glutathione. Sensors Actuators. B Chem. 2013; 184: 15-20.
2. Keyvanfard M, Jalilian Z, Alizad K. Voltammetric determination of glutathione in pharmaceutical and biological samples using multiwall carbon nanotubes paste electrode in the presence of rutin as a mediator. IJPR. 2016;15:1-20.
3. Li X, Huo F, Yue Y, Zhang Y, Yin C. A coumarin-based "off-on" sensor for fluorescence selectivily discriminating GSH from Cys/Hcy and its bioimaging in living cells. Sensors Actuators, B Chem. 2017;253:42-9.
4. Muthirulan P, Velmurugan R. Direct electrochemistry and electrocatalysis of reduced glutathione on CNFs-PDDA/PB nanocomposite film modified ITO electrode for biosensors. Colloids Surfaces B. 2011;83:347-54.
5. Raoof JB, Teymoori N, Khalilzadeh MA, Ojani R. A high sensitive electrochemical nanosensor for simultaneous determination of glutathione, NADH and folic acid. Mater Sci Eng C. 2015; 47: 77-84.
6. Heng S, Zhang X, Pei J, Abell AD. A Rationally designed reversible "turn-off" sensor for glutathione. Biosensors. 2017;7:1-13.
7. Niu WJ, Zhu RH, Cosnier S, Zhang XJ, Shan D. Ferrocyanide-Ferricyanide Redox Couple Induced Electrochemiluminescence Amplification of Carbon Dots for Ultrasensitive Sensing of Glutathione. Anal Chem. 2015;87:11150-11156.
8. Rojas L, Molero L, Tapia RA, Rio R, Valle MA, Antilén M, et al. Electrochemistry behavior of endogenous thiols on fluorine doped tin oxide electrodes. Electrochim Acta. 2011; 56(24): 8711-8717.
9. Turan K, Sarikaya E, Demir H, Demir C. Investigation of prolidase, adenosine deaminase, glutathione s-transferase and glutathione reductase activities in patients with abortus imminens. JCAM. 2017;8:519-522.
10. Yang C, Wang X, Liu H, Ge S, Yu J, Yan M. On–off–on fluorescence sensing of glutathione in food samples based on a graphitic carbon nitride ($g\text{-}C_3N_4$)–Cu^{2+} strategy. New J Chem. 2017;41:3374-3379.
11. Yu Y, Shi J, Zhao X, Yuan Z, Lu C, Lu J. Electrochemiluminescence detection of reduced and oxidized glutathione ratio by quantum dot-layered double hydroxide film. Analyst. 2016;141:3305-3312.
12. Karimi-Maleh H, Bananezhad A, Ganjali MR, Norouzi P. Electrochemical nanostructure platform for the analysis of glutathione in the presence of uric acid and tryptophan. Anal Methods. 2017;9(44):6228-6234.
13. Zhang B, Liu J, Ma X, Zuo P, Ye BC, Li Y. Ultrasensitive and selective assay of glutathione species in arsenic trioxide-treated leukemia HL-60 cell line by molecularly imprinted polymer decorated electrochemical sensors. Biosens Bioelectron. 2016;80:491-6.
14. Kizek R, Vacek J, Trnková L, Jelen F. Cyclic voltammetric study of the redox system of glutathione using the disulfide bond reductant tris (2-carboxyethyl) phosphine. Bioelectrochemistry. 2004;63(1-2):19-24.
15. Dusak A, Atasoy N, Demir H, Doğan E, Gürsoy T, Sarıkaya E. Investigation of levels of oxidative stress and antioxidant enzymes in colon cancers Kolon kanserlilerde oksidatif stres ve antioksidan enzim seviyelerinin incelenmesi. JCAM. 2017;8(6):469-73.
16. Wang Y, Lu J, Tang L, Chang H, Li J. Graphene oxide amplified electrogenerated chemiluminescence of quantum dots and its selective sensing for glutathione from thiol-containing compounds. Anal Chem. 2009;81(23):9710-9715.
17. Niu X, Zhao H, Lan M, Zhou L. Platinum nanoparticles encapsulated in carbon microspheres: Toward electro-catalyzing glucose with high activity and stability. Electrochim Acta. 2015;151:326-31.
18. Rostami A, Taylor MS. Polymers for anion recognition and sensing. 3rd ed. New York: Taylor & Francis Group. 2012.
19. Jürgen D, Steckhan E. Influence of the supporting electrolyte and the pH on the electrooxidative activation of glassy carbon electrodes. J Electroanal Chem. 1992;333:177-93.
20. Owen JB, Butterfield DA. Measurement of Oxidized/Reduced Glutathione Ratio. vol. 648. Humana Press. Totowa: NJ; 2010.
21. Zitka O, Skalickova S, Gumulec J, Masarik M, Adam V, Hubalek J, et al. Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumour patients. Oncol Lett. 2012;4:1247-53.
22. Chee SY, Flegel M, Pumera M. Regulatory peptides desmopressin and glutathione voltammetric determination on nickel oxide modified electrodes. Electrochem. Commun. 2011;13:963–5.
23. Pang H, Shi YF, Du JM, Ma YH, Li GC, Chen J, et al. Porous nickel oxide microflowers synthesized by calcination of coordination microflowers and their applications as glutathione electrochemical sensor and supercapacitors. Electrochim Acta. 2012;15:256–62.
24. Raoof JB, Ojani R, Baghayeri M. Simultaneous electrochemical determination of glutathione and tryptophan on a nano-TiO2/ferrocene carboxylic acid modified carbon paste electrode. Sensors Actuators, B Chem. 2009;143: 261–9.
25. Hou Y, Ndamanisha JC, Guo LP, Peng XJ, Bai J. Synthesis of ordered mesoporous carbon/cobalt oxide nanocomposite for determination of glutathione Electrochim Acta. 2009;54:6166–6171.
26. Ndamanisha JC, Bai J, Qi B, Guo LP. Application of electrochemical properties of ordered mesoporous carbon to the determination of glutathione and cysteine. Anal Biochem. 2009;386:79–84.
27. Wang XJ, Chen X, Evans DG, Yang WS. A novel biosensor for reduced l-glutathione based on cobalt phthalocyanine tetrasulfonate-intercalated layered double hydroxide modified glassy carbon electrodes. Sensors Actuators, B Chem. 2011;160:1444–1449.
28. Xu F, Wang L, Gao MN, Jin LT, Jin JY. Amperometric determination of glutathione and cysteine on a Pd-IrO2 modified electrode with high performance liquid chromatography in rat brain microdialysate. Anal Bioanal Chem. 2002;372:791–4.
29. Pereira-Rodrigues N, Cofré R, Zagal JH, Bedioui F. Electrocatalytic activity of cobalt phthalocyanine CoPc adsorbed on a graphite electrode for the oxidation of reduced l-glutathione (GSH) and the reduction of its disulfide (GSSG) at physiological pH. Bioelectrochem. 2007;70:147–54.
30. Inoue T, Kirchhoff JR. Electrochemical detection of thiols with a coenzyme pyrroloquinoline quinone modified electrode. Anal Chem. 2000;23:5755–5760.
31. Eremenko AV, Dontsova EA, Nazarov AP, Evtushenko EG, Amitonov SV, Savilov SV et al. Manganese dioxide nanostructures as a novel electrochemical mediator for thiol sensors. Electroanalysis. 2012;24:573–80.
32. Chatraei F, Zare HR. Electrodeposited acetaminophen as a bifunctional electrocatalyst for simultaneous determination of ascorbic acid, glutathione, adrenaline and tryptophan. Analyst. 2011;136:4595–4602.

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